

#### Figure S1. Summer gene expression pattern clusters (Related to Figure 1)

Normalized expression of 16L:8D-induced transcripts (rDEI<sub>8L:16D/16L:8D</sub> < 0.5) grouped by k-means clustering. 16L:8D (red) and 8L:16D (blue) expression patterns were transformed to Z score together for clustering to retain relative magnitude. Black lines indicate median expression level. Grey rectangles indicate the dark period of each photoperiod. The number of clusters is determined by the elbow method. Top enriched Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.



### Figure S2. *PP2-A13* is needed for proper development and fitness in 8L:16D (Related to Figure 2)

(A) Schematic shows the T-DNA insertion site in *PP2-A13*. Black boxes = exons; black lines = non-coding sequences.

(B) qRT-PCR of full length, 5' end, and 3' end of the *PP2-A13* gene. Tissue was collected at ZT11 from 12-day-old plants grown in 8L:16D. *UBQ10* was used as an internal control. n = 3 samples containing multiple seedlings. Error bar indicates SD. \*,  $p \le 0.05$  (Welch's t-test).

(C) Aerial fresh weight of wild-type (Col) and *pp2-a13-1* mutant plants grown in 16L:8D and 8L:16D were normalized to the mean of wild-type (Col) at each time point. Black (Col wild type) or orange (*pp2-a13-1* mutant) lines indicate the mean of each genotype at different time points. *n* = 3-8 individual plants. Asterisks indicate significant difference between wild-type (Col) and *pp2-a13-1* mutant plants at each time point. \*, *p*≤0.05; \*\*, *p*≤0.01; \*\*\*\*, *p*≤0.001; \*\*\*\*, *p*≤0.0001 (Welch's t-test).

(D) Leaf phenotypes of 80-day-old *pp2-a13-1* mutant and wild type (Col) grown in 8D:16D.

(E) Representative images of wild-type (Col) and *pp2-a13-1* mutant plants at different time points prior to flowering. Plants were grown in 16L:8D and 8L:16D. Scale bar = 2 cm in 16L:8D and 3 cm in 8L:16D.

(F) Number of days until appearance of 1 cm long bolt for wild-type (Col) and *pp2-a13-1* mutant plants grown in 16L:8D and 8L:16D. n= 52-60. \*\*\*\*,  $p\leq$ 0.0001 (Welch's t-test).

(G) Number of days until anthesis of the first flower for wild-type (Col) and *pp2-a13-1* mutant plants grown in 16L:8D and 8L:16D. n = 52-60. Welch's t-test was performed on values excluding the four non-anthesed plants. \*\*\*\*,  $p \le 0.0001$ .

(H) Segregating progeny from *PP2-A13<sub>promoter</sub>::gPP2-A13* complementation lines in the *pp2-a13-1* mutant background. +/+ and +/- indicate homozygous and hemizygous for the transgene, respectively. Images were taken of 9-week-old plants grown in 8L:16D. Scale bar = 3 cm.



### Figure S3. PP2-A13 expression is controlled by photoperiod (Related to Figure 4)

- (A) Representative images and intensity changes for data presented in figure 4E.
- (B) *PP2-A13*<sub>promoter</sub>::Luciferase expression in plants grown under short day conditions with either 100  $\mu$ M m<sup>-2</sup> s<sup>-1</sup> (blue) or 200  $\mu$ M m<sup>-2</sup> s<sup>-1</sup> (teal) white light.
- (C) Intensity change calculations for data presented in figure 4F.
- (D) Representative images, normalized traces, and intensity changes for traces presented in figure 4H.
- (E) Intensity changes for data presented in figure 4I.
- (F) Representative images, normalized intensity, and intensity changes for figure 4J.
- (G) Representative images, normalized intensity, and intensity changes for figure 4K.



#### Figure S4. PP2-A13 critical photoperiod (Related to Figure 4)

(A) Data is same as in Figure 4F except plotted independently for clarity.

(B-C) *PP2-A13<sub>promoter</sub>::Luciferase* expression in plants grown under 4L:20D conditions (B, purple) and 20L:4D conditions (C, magenta).

(D) Curve fit for estimated yearly expression of *PP2-A13<sub>promoter</sub>::Luciferase*. Approximately sigmoidal fit to the total, normalized intensity of *PP2-A13<sub>promoter</sub>::Luciferase* in a day. Blue points are the experimental points from the 6 conditions in figure S4A. Red line is the approximate sigmoidal fit.



# Figure S5. Circadian clock, not CONSTANS, regulates *PP2-A13* expression in photoperiod flip experiments (Related to Figure 5)

*PP2-A13*<sub>promoter</sub>::Luciferase traces from co-9, elf3-1, and lux-4 mutant plants grown under 8L:16D conditions and transferred into 16L:8D conditions on day 11 (A), or grown under 16L:8D conditions and transferred into 8L:16D conditions on day 11 (B).



## Figure S6. The photosynthetic apparatus senses darkness for winter photoperiod time measurement (Related to Figure 6)

- (A) Representative images, normalized traces, and intensity changes for traces presented in figure 6A.
- (B) Representative images, normalized traces, and intensity changes for traces presented in figure 6B.
- (C) Representative images, normalized traces, and intensity changes for traces presented in figure 6C.





PP2-A13

L:8D to 8L

### Figure S7. Starch mutants have defects in *PP2-A13* photoperiodic gene expression (Related to Figure 7)

(A, C, E) *PP2-A13<sub>promoter</sub>::Luciferase* trace data from wild-type plants grown in 8L:16D (A), 16L:8D (C), and double dusk (8L:4D:8L:4D) (E) conditions.

(B, D, and F) Normalized traces of the daily luciferase intensity pattern from wild-type (figure S7A, S7C, and S7E), *pgm-1* (figure 7A-C, pink traces), and *sex1-1* (figure 7A-C, green traces) mutant plants.

(G-H) *PP2-A13<sub>promoter</sub>::Luciferase* trace data from *pgm-1* and *sex1-1* mutant plants grown in 16L:8D with 90 mM sorbitol or 90 mM sucrose.

(I-J) *PP2-A13*<sub>promoter</sub>::Luciferase traces from *pgm-1* and *sex1-1* mutant plants grown under 8L:16D conditions and transferred into 16L:8D conditions on day 11 (I), or grown under 16L:8D conditions and transferred into 8L:16D conditions on day 11 (J).

(K) Model for winter photoperiodism in plants. In the light, photosynthesis produces sucrose, which represses *PP2-A13* and other winter photoperiod genes. In the dark, photosynthesis ceases and starch breakdown controls sucrose levels across the night. The circadian clock regulates the rate of starch breakdown, restricting production of sucrose in long winter-like nights, conversely promoting breakdown in short summer-like nights. Low post-dusk levels of sucrose in winter photoperiods allow *PP2-A13* and other winter photoperiod genes to be rapidly induced. These genes are involved in energy conservation, dormancy, nutrient recycling, flowering repression, and other important cellular processes for wintertime. High post-dusk levels of sucrose in summer photoperiods represses *PP2-A13* and other winter photoperiod genes.

Primer name	Primer sequence (5' to 3')
Cloning primers	
PP2-A13-F	CACCATGGGCGCGAATATCTCGGGAG
PP2-A13-R-NS	TTGTGATCCAATGACTTCT
PP2-A13pro-F	CACCGTAAACTTTGGGTTTAAGCAAGA
PP2-A13pro-R	CAAACAAAACGAAGACCCTAAA
Genotyping primers	
atg5-1-LP	ATTTGCTATTTGTTTGGCACG
atg5-1-RP	TACCGTTCATGACAGAGGTCC
atg7-2-LP	CGTGTAACAGTGCATTGTTGG
atg7-2-RP	GGAGCTTAACAAAGGGAAACG
co-9-LP	AAGCTGTTGTGACACATGCTG
co-9-RP	CCCCTTCTTTCAGATACCAGC
LBb1.3	ATTTTGCCGATTTCGGAAC
pgm-1-gF	CCACTTTGTTGACATATCGAG
pgm-1-gR	GTGTACCAGAGACGAGACC
pGWB-Rev	GTTTGAACGATCGGGGAAA
pp2-a13-1-LP	CACGAGTATATGTATGTACACACATC
pp2-a13-1-RP	CTATTGTGATCCAATGACTTCT
SAIL-LB3	TAGCATCTGAATTTCATAACCAATCTCGATACAC
sex1-1-gF	TGAAGGTGAACAGAGATGGGAGC
sex1-1-gR	TCACACTTGTGGTCGTGTCTGG
elf3-1-gF	CGATGCAGAATTATAGTTTCTTC
elf3-1-gR	TTAAGGCTTAGAGGAGTCATAGCG
lux-4-gF	AGATCGAGTTGGATCTAGCAGTCC
lux-4-gR	CATTTCCACCAGCTCCATGATGATG
qRT-PCR primers	
3'-PP2-A13-qF	GAACAACAACCCTGGGAGCT
3'-PP2-A13-qR	GATCCAATGACTTCTTTTGCACA
5'-PP2-A13-qF	GGTTCACCGGAGTTTGACC
5'-PP2-A13-qR	GGTGGATCAAGCCGTGTCAT
ATG8a-qF	GCAGAGACTAATCGAATCGC
ATG8a-qR	CAAGCAACGGTAAGAGATCC
IPP2-qF	ATTTGCCCATCGTCCTCTGT
IPP2-qR	GAGAAAGCACGAAAATTCGGTAA
PP2-A13-qF	TGAATCCAGGTTCCAGTCAGC
PP2-A13-qR	TGCAGATCCTCCTTCCAAGC

Table S5: Primers used in this study (Related to Figure 2, 3, 6; S2; STAR★Methods )

UBQ10-qF	AGAAGTTCAATGTTTCGTTTCATGTAA
UBQ10-qR	GAACGGAAACATAGTAGAACACTTATTCA